Short Communication

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Replicon Typing of O2, O6, and O157 Serotypes of Escherichia Coli Using PCR Method Based on Plasmid Incompatibility Groups

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ABSTRACT

BACKGROUND AND OBJECTIVE: Today, the PCR-based replicon typing of major plasmid incompatibility groups is used in epidemiological studies to describe the distribution of traits such as antibiotic resistance in the bacteria of *enterobacteriaceae* family. Despite the crucial role of plasmids in transmitting pathogens, little studies have been conducted on the type of plasmid transmitted by bacterial serotypes of *E. coli*. The main objective of this study was to classify plasmid O2, O6 and O157 serotypes of *E. coli* bacteria based on major plasmid incompatibility groups using multiplex-PCR method.

METHODS: This descriptive study was performed on 40 isolates of *E. coli* related to O6, O2 and O157 serotypes to identify 8 plasmid replicons (*IncN, IncFIB, FreP, IncB/O, Inc FIIA, IncFIC, IncFIA, IncI1*) by multiplex-PCR method.

RESULTS: The results showed that the amplicon of Fre (88%), and replicons of IncFIB (78%), IncFIA (60%), IncB/O (53%), IncI1 (50%), IncN (25%) were observed in 40 studied isolates. IncFIC and IncFIIA replicons were not observed in any of the isolates.

CONCLUSION: The results of this study indicated a high diversity of plasmid incompatibility groups among the studied isolates.

KEY WORDS: *Plasmid, Multiplex-PCR, Replicon.*

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Introduction

Naturally, bacterial plasmids are self-propagating and diverse elements that encode traits such as resistance to antibiotics and heavy metals, durability and adaptation in the environment for bacteria (1, 2). Plasmids are also the main source of horizontal gene transfer (HGT), which plays an important role in adapting bacteria to environmental changes (3). Additionally, plasmids can contribute to the dynamics of the bacterial genome through the transfer of transferable elements and transposons and their incorporation into the chromosome of the bacterium (a homogeneous or non-homogeneous recombinant)(1). Due to their role in HGT (1), especially in regard with the emergence and release of antimicrobial resistance (4, 5), much attention has been paid to the identification and classification of bacterial plasmids.

Typically, plasmids are classified based on their incompatibility (traits related to duplication or replication of plasmids) (6). Plasmid incompatibility (Inc) typing is based on the fact that two plasmids that have the same sequence of DNA replication and isolation cannot replicate in one host (6). Since plasmid incompatibility (Inc) typing is based on their replication factors, the terms Inc and Rep are used to describe the types of plasmids in a host (1, 6, 7). Currently, 27 incompatibility (Inc) groups have been identified among the Enterobacteriaceae family. Classification of plasmids based on incompatibility groups is very desirable due to their role in antibiotic resistance and transmission of pathogenic factors (2,5,8–10). Unfortunately, conducting a study based on physical incompatibility, especially when applied to large bacterial populations, is tedious and time-consuming. In 1988, Couturier et al. developed a hybridization method to compare bacterial plasmids based on their replication type from 19 probes corresponding to 19 different incompatibility groups.

Although this method represents a significant improvement in the typing of plasmids, the timely, tedious and incompatible method was the preferred method for current approaches (6). In a study by Carattoli et al., a simple and practical PCR-based replicon typing method was used to classify 18 plasmid incompatibilities in the Enterobacteriaceae family (8). Although this method was much simpler than the previous ones, however, due to having multiplex-PCR step and three simple PCR steps, it can be tedious and costly to study the plasmids of large bacterial populations, and the same method was used in the present study. Extraintestinal Escherichia coli causes various diseases in humans and animals. This group typically has plasmids associated with pathogenicity and antimicrobial resistance (2, 11, 12). This is true about the strains of Uropathogenic Escherichia coli causing urinary tract infections or the UroPathogenic Escherichia coli (UPEC), which cause cystitis and nephritis in humans and animals, which has a large number of plasmids. Recently, it has been reported by researchers that the presence of ColV and ColBM in the extraintestinal Escherichia coli is a well-defined characteristic of their pathotyping.

UPEC strains are the leading cause of urinary tract infections, including cystitis and pyelonephritis. In these bacteria, the O–serogroups belong to the virulence profile in each strain. Previous studies have shown that serogroups O_1, O_2, O_4, O_6, O_7, O_8, O_{15}, O_{16}, O_{18}, O_{21}, O_{22}, O_{25}, O_{75}, and O_{83} are respectively associated with UPEC strains, and the prevalence of different serotypes varies in different regions (13). Research has shown that the plasmids found in these bacteria belong to the IncFIB incompatibility group, which in its genome has highly protected areas, such as FIB replicon, ColV or ColBM operon, and several operons containing pathogenic genes and genes involved in acquiring and transferring iron. In addition, these types of plasmids and other plasmids that encode antibiotic resistance are found in UPEC bacteria (2, 7, 14, 15).

Considering the prevalence of urinary tract infections and increased antibiotic resistance in different human populations, and due to negligence in treating these types of infections, irreparable problems will occur in the future. Therefore, screening, identification and classification of plasmids are important for epidemiological studies for proper control and treatment of urinary tract infections. Therefore, the present study was conducted to determine the serotypes of O2, O6, and O157 of *Escherichia coli* isolated from urinary tract infections based on plasmid replication using multiplex-PCR method.

Methods

This prospective study was conducted on 40 *E. coli* isolates related to O_2, O_6, and O_{157} serotypes to determine the presence of 8 plasmids replicon (IncN, IncFIB, FreP, IncB/O, Inc FIIA, IncFIC, IncFIA and IncI1) by multiplex-PCR method. Ethical approval was granted by the Zabol university research ethics committee (IR–OUZ–93.08) and all *E.coli* isolates were collected in previously study from patients with urinary tract infection (16,17).

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Genomic DNA extraction: First, *E. coli* isolates were grown in Luria-Bertani (LB) Broth (HiMedia, India) at 37°C for 18 – 24 hours in a shaking incubator (Labnet International Inc.). Bacteria were pelleted from the liquid medium using centrifuge (Eppendorf, Germany) and were washed two times with 1% PBS solution and resuspended in 200 uL of sterile distilled water, then incubated at 100°C for 10 minutes and centrifuged. One hundred microliters of the supernatant was stored at -20°C as a template DNA stock.

Detection of the presence of plasmid replicon: specific primers were used to amplify sequences of the plasmid replicon (N, FIB, FreP, B / O, FIIA, FIC, FIA, and I1) in *E.coli* isolates. Details of primer sequences, predicted sizes of the amplified products, and specific annealing temperatures are shown in Table 1. Detection of plasmid replicon was done by multiplex PCR in three PCR panel: Panel 1 (B/O, and FIC), Panel 2 (FIB, FIA, and FIIA), and Panel 3 (FreP, I1, and N) (7). The reactions (15 µL) consisted of 1 µL of the Forward and Reverse primers (20 pmol/µL), 2 µL templates DNA, and 8 µL of a ready-to-use 2X PCR Master Mix Red (Ampliqon; Denmark) and 4 µl of double distilled water, with the following amplification conditions: an initial denaturation at 94°C for 5 minutes, followed by 30 DNA cycles of denaturation at 94°C for 30 seconds, annealing at 58 °C for 30 seconds (Table 1), and extension at 72°C for 90 seconds and final extension for 5 minutes at 72°C. A 5 µL aliquot of the PCR product underwent gel electrophoresis on agarose 2%, followed by staining with ethidium bromide solution.

Amplified DNA elements of specific sizes were detected by UV-induced fluorescence and the size of the amplicons was estimated by comparing them with the 100 bp DNA Ladder (Fermentas) included on the same gel (Fig 1).

Table 1. Sequence of the primers of plasmid replicons

Plasmid replicon	Primer	Primer sequence	N(%)	Size (bp)	Temperature	Source
B/O	F	GCGGTCCGGAAAGCCAGAAAAC	22	159	60	7
	R	TCTGCGTTCCGCCAAGTTCGA	21		60	
FIC	F	GTGAACTGGCAGATGAGGAAGG	22	262	60	7
	R	TTCTCCTCGTCGCCAAACTAGAT	23		60	
FIIA	F	CTGTCGTAAGCTGATGGC	18	270	60	7
	R	CTCTGCCACAAACTTCAGC	19		60	
FIA	F	CCATGCTGGTTCTAGAGAAGGTG	23	462	60	7
	R	GTATATCCTTACTGGCTTCCGCAG	24		60	
FIB	F	GGAGTTCTGACACACGATTTTCTG	24	702	60	7
	R	CTCCCGTCGCTTCAGGGCATT	21		60	
I1	F	CGAAAGCCGGACGGCAGAA	19	139	60	7
	R	TCGTCGTTCCGCCAAGTTCGT	21		60	
Frep	F	TGATCGTTTAAGGAATTTTG	20	270	60	7
	R	GAAGATCAGTCACACCATCC	20		60	
N	F	GTCTAACGAGCTTACCGAAG	20	559	60	7
	R	GTTTCAACTCTGCCAAGTTC	20		60	

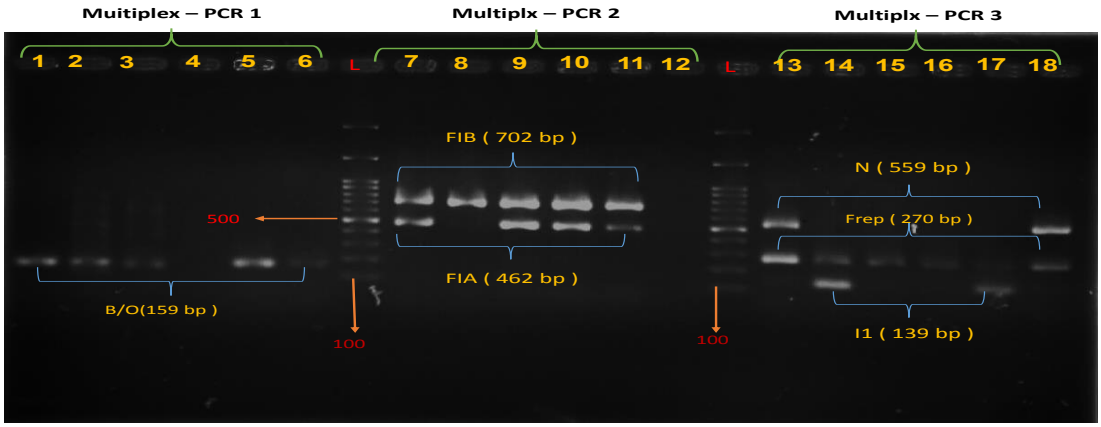


Figure 1. Multiplex- PCR profiles specific for detection of plasmid replicon in *E.coli* isolates. Lane1 to 6 are related to B / O plasmid replicon with 159 bp. Lane 7 to 12 are related to FIA and FIB plasmids with 462 and 702 bp. Lane 4 is related to FIB plasmid replicon with 702 bp. Lane 5 is related to FIA and FIB plasmid replicon with 462 and 702 bp. Lane 6 is related to plasmids I1 and N with 139 and 270 bp. Lane 7 is related to Frep and N plasmid replicons with 270 and 559 bp and Lane 8 is related to I1, N and Frep plasmid replicons with 139, 270 and 559 bp, respectively.

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Results

A total of 40 E. coli isolates belonging to the O2, O6 and O157 serotypes were classified based on their plasmid replicons. Of the 40 studied isolates, the Frep plasmid replicon with 88% had the highest frequency, and the IncN plasmid replicon with 25% had the lowest frequency. In addition, IncFIC and IncFIIA replicons were not found in any of the samples.

The frequency of IncFIB, IncFIA, IncB/O, IncI1 plasmid replicons in the 40 isolates were 50%, 53%, 60%, and 78%, respectively (Fig 2). Moreover, IncN, IncI1, and IncB/O replicons in serotype O157, Frep replicon in serotype O6, and IncFIA and IncFIB replicons in the serotype O2 were the most frequent. There was no significant relationship between serotype and type of plasmid (Fig3).

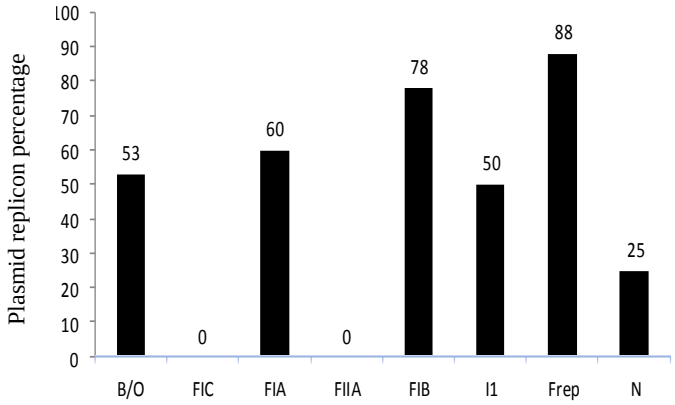


Figure 2. Prevalence (%) of Plasmid replicon among 40 *E. coli* isolates

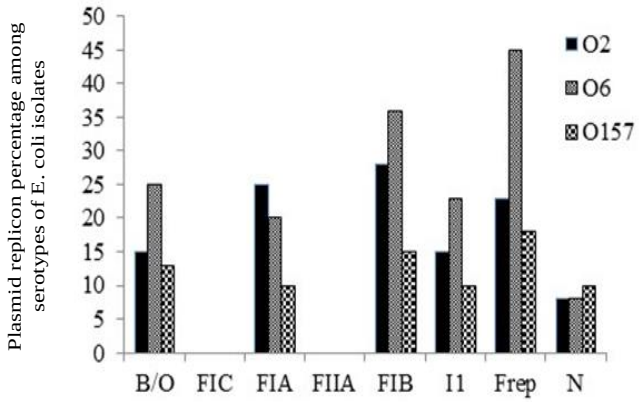


Figure 3. Prevalence (%) of Plasmid replicon among studied serotypes of *E. coli* isolates

Discussion

This study showed that 88% of isolates containing Frep replicon and Inc N, Inc I1, IncB / O, IncFIA and IncFIB replicons were observed in 25%, 50%, 53%, 60%, and 78% of isolates, respectively. In a similar study conducted by Johnson et al. on UTI and fecal E.coli isolates, Frep replicon had the highest frequency and Inc N replicon had the lowest frequency, which is consistent with the present study (7).

IncFIC and IncFIIA were also not found in any of the studied isolates. Compared to the data reported by Abraham et al and consistent with our results, IncFIC and IncFIIA replicons were not found in any of the isolates (18). Of note, within our collection we found a prevalence of the IncF (IncFIA and IncFIB) replicon with 24% and 31% respectively which found more frequent than other studied groups. Similar results have been reported by Lyimo et al. that IncF type was found in most of the isolates (19). This group also showed that the IncF plasmids were attributable to the highest conjugation efficiency (19).

In addition, Yang et al. demonstrated that IncF is the most prevalent plasmid among E. coli isolates (20). This group also reported that most of IncF plasmids harbored multiple antibiotic resistance genes, indicating their importance in distribution of antibiotic resistance among bacterial populations. In addition, these plasmids have an addiction system that helps them maintain their stability in bacterial host under different conditions (20), which may be the reason for the high frequency of this group of plasmids in *E. coli* strains. Our results also indicated that 50% of studied isolates were positive for $IncI_1$ group indicates its importance in the transmission of the antibiotic resistance genes among the *E. coli* population (21).

In a study, Xia et al. reported that IncI1 plasmid carries antibiotic resistance genes that can remain in human and animal gut flora for a long time and become a reservoir for the continuous transmission of these genes among bacterial species (22). In the present study the replicons were 53% and 25% for IncB / O and IncN respectively. In addition various studies have shown that broad-spectrum β-lactamase genes is dominantly harbored by IncI1, A / C or Inc N in animals and humans (23-25). In previously performed study by Abdi et al on the isolates of this study, showed that 92%, 74% and 71% of the isolates were resistant to ampicillin, ceftizoxime and cefixime respectively (26).

The results of this study showed that the presence of IncI1, A/C or Inc N plasmids in these isolates may be a major cause of resistance to these antibiotics. Our analysis showed that 1 isolate had 6 plasmids, 7 isolates had 5 plasmids and 14 isolates had 4 plasmids, indicating a high diversity of plasmid in the studied

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isolates that is consistent with studies conducted by Hopkines et al and Zurfluh et al that shows a diversity of plasmids that carry genes among Escherichia coli and Salmonella enterica originating from animals and humans (5, 27). Since the PBRT molecular technique is a simple and quick method for the detection and classification of the incompatibility plasmids of E. coli strains, this study was conducted in Iran for the first time in the Sistan region to track the evolution and development of E. coliisolates collected from urinary tract infections. This study is also used to find out more about the pathogenesis of infections, the management of infectious diseases and epidemiological studies, and it is suggested that similar studies be carried out extensively in other parts of Iran.

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References

1.Frost LS, Leplae R, Summers AO, Toussaint A. Mobile genetic elements: the agents of open source evolution. Nat Rev Microbiol. 2005;3(9):722-32.

2.Johnson TJ, Giddings CW, Horne SM, Gibbs PS, Wooley RE, Skyberg J, et al. Location of increased serum survival gene and selected virulence traits on a conjugative R plasmid in an avian Escherichia coli isolate. Avian Dis. 2002;46(2):342-52.

3.Smets BF, Barkay T. Horizontal gene transfer: perspectives at a crossroads of scientific disciplines. Nat Rev Microbiol. 2005;3(9):675-8.

4.Bertini A, Poirel L, Mugnier PD, Villa L, Nordmann P, Carattoli A. Characterization and PCR-based replicon typing of resistance plasmids in Acinetobacter baumannii. Antimicrob Agents Chemother. 2010; 54(10):4168-77.

5.Hopkins KL, Liebana E, Villa L, Batchelor M, Threlfall EJ, Carattoli A. Replicon typing of plasmids carrying CTX-M or CMY beta-lactamases circulating among Salmonella and Escherichia coli isolates. Antimicrob Agents Chemother. 2006;50(9):3203-6.

6.Couturier M, Bex F, Bergquist PL, Maas WK. Identification and classification of bacterial plasmids. Microbiol Rev. 1988;52(3):375-95.

7.Johnson TJ, Wannemuehler YM, Johnson SJ, Logue CM, White DG, Doetkott C, et al. Plasmid replicon typing of commensal and pathogenic Escherichia coli isolates. Appl Environ Microbiol. 2007;73(6):1976-83.

8.Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods. 2005;63(3):219-28.

9.Boyd D, Cloeckaert A, Chaslus-Dancla E, Mulvey MR. Characterization of variant Salmonella genomic island 1 multidrug resistance regions from serovars Typhimurium DT104 and Agona. Antimicrob Agents Chemother. 2002;46(6):1714-22.

10.Gilmour MW, Thomson NR, Sanders M, Parkhill J, Taylor DE. The complete nucleotide sequence of the resistance plasmid R478: defining the backbone components of incompatibility group H conjugative plasmids through comparative genomics. Plasmid. 2004;52(3):182-202.

11.Dobrindt U. (Patho-)Genomics of Escherichia coli. Int J Med Microbiol. 2005;295(6-7):357-71.

12.Klumpp DJ, Weiser AC, Sengupta S, Forrestal SG, Batler RA, Schaeffer AJ. Uropathogenic Escherichia coli potentiates type 1 pilus-induced apoptosis by suppressing NF-kappaB. Infect Immun. 2001;69(11):6689-95.

13.Sharma S, Kaur N, Malhotra S, Madan P, Ahmad W, Hans C. Serotyping and Antimicrobial Susceptibility Pattern of Escherichia coli Isolates from Urinary Tract Infections in Pediatric Population in a Tertiary Care Hospital. J Pathogens. 2016;2016:2548517.

14.Johnson TJ, Johnson SJ, Nolan LK. Complete DNA sequence of a Col BM plasmid from avian pathogenic Escherichia coli suggests that it evolved from closely related ColV virulence plasmids. J Bacteriol. 2006;188(16):5975-83.

15.Johnson TJ, Jordan D, Kariyawasam S, Stell AL, Bell NP, Wannemuehler YM, et al. Sequence analysis and characterization of a transferable hybrid plasmid encoding multidrug resistance and enabling zoonotic potential for extraintestinal Escherichia coli. Infect Immun. 2010;78(5):1931-42.

16.Rashki A, Abdi HA, Shookohi M. Prevalence of Genes Encoding Outer Membrane Virulence Factors Among Fecal Escherichia coli Isolates. Int J Basic Sci Med. 2017;2(1):52-7.

17.Rashki A, Abdi HA. O-serotyping of Escherichia coli Strains isolated from Patients With Urinary Tract Infection in Southeast of Iran. Int J Enteric Pathog. 2014;2(4):e20968.

18.Abraham S, Chapman TA, Zhang R, Chin J, Mabbett AN, Totsika M, et al. Molecular characterization of Escherichia coli strains that cause symptomatic and asymptomatic urinary tract infections. J Clin Microbiol. 2012;50(3):1027-30.

19.Lyimo B, Buza J, Subbiah M, Temba S, Kipasika H, Smith W, et al. IncF Plasmids Are Commonly Carried by Antibiotic Resistant Escherichia coli Isolated from Drinking Water Sources in Northern Tanzania. Int J Microbiol. 2016;2016:3103672.

20.Yang QE, Sun J, Li L, Deng H, Liu BT, Fang LX, et al. IncF plasmid diversity in multi-drug resistant Escherichia coli strains from animals in China. Front Microbiol. 2015;6:964.

[DOR: 20.1001.1.15614107.1397.20.7.6.6]
[DOI: 10.18869/acadpub.jbums.20.7.40]

21.Johnson TJ, Shepard SM, Rivet B, Danzeisen JL, Carattoli A. Comparative genomics and phylogeny of the IncI1 plasmids: a common plasmid type among porcine enterotoxigenic Escherichia coli. Plasmid. 2011;66(3):144-51.
22.Xia L, Liu Y, Xia S, Kudinha T, Xiao SN, Zhong NS, et al. Prevalence of ST1193 clone and IncI1/ST16 plasmid in E-coli isolates carrying blaCTX-M-55 gene from urinary tract infections patients in China. Sci Rep. 2017;7:44866.
23.Cloeckaert A, Praud K, Doublet B, Bertini A, Carattoli A, Butaye P, et al. Dissemination of an extended-spectrum-beta-lactamase blaTEM-52 gene-carrying IncI1 plasmid in various Salmonella enterica serovars isolated from poultry and humans in Belgium and France between 2001 and 2005. Antimicrob Agents Chemother. 2007;51(5):1872-5.
24.Cloeckaert A, Praud K, Lefevre M, Doublet B, Pardos M, Granier SA, et al. IncI1 plasmid carrying extended-spectrum-beta-lactamase gene blaCTX-M-1 in Salmonella enterica isolates from poultry and humans in France, 2003 to 2008. Antimicrob Agents Chemother.54(10):4484-6.
25.Garcia-Fernandez A, Chiaretto G, Bertini A, Villa L, Fortini D, Ricci A, et al. Multilocus sequence typing of IncI1 plasmids carrying extended-spectrum beta-lactamases in Escherichia coli and Salmonella of human and animal origin. J Antimicrob Chemother. 2008;61(6):1229-33.
26.Abdi HA, Rashki GM. Virulence Genes, Genetic Diversity, Antimicrobial Susceptibility and Phylogenetic Background of Escherichia coli Isolates. Int J Enteric Pathog. 2015;3(3):e25692.
27.Zurfluh K, Jakobi G, Stephan R, Hachler H, Nuesch-Inderbinen M. Replicon typing of plasmids carrying bla CTX-M-1 in Enterobacteriaceae of animal, environmental and human origin. Front Microbiol. 2014;5:555.

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